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100-92-17

Hyperbolic Kussner

NEW METHODS AND USE

TECHNICAL FIELD

This invention relates to methods and uses of the integrin alpha10 chain, for preventing progression of atherosclerosis plaque formation in a mammal. Also included is a method and use of the integrin alpha10 chain for detecting atherosclerotic plaque in a mammal.

BACKGROUND OF THE INVENTION

10

Atherosclerosis

Atherosclerosis and its thrombotic complications are the major cause of morbidity and mortality in industrialised countries. The number of prevalent atherosclerotic cases in 2000 totalled nearly 174 million in the major pharmaceutical markets and this figure will continue to increase with the increase in the ageing population. Still, only a fraction of these show overt signs of the disease. The rest remain undiagnosed until the disease manifests itself symptomatically, in the worst case as a heart attack or stroke.

Atherosclerosis is a focal pathological phenomenon characterised by the thickening and hardening of arteries due to the accumulation of lipids (mainly cholesterol esters), carbohydrates, blood products, fibrous tissue and calcium within the vessel wall beginning with the subendothelial space. The gradual build-up of fatty deposits leads to the formation of plaques which eventually narrow and block the artery. The causes and detection of such plaque formation has been intensely investigated.

Atherosclerosis can occur in any artery. In coronary arteries, it may result in heart attacks; in cerebral arteries it may result in strokes; in peripheral arteries it may result in gangrene of the extremities.

The American Heart Association Committee on Vascular Lesions (Stary *et al*, Arterioscler Thromb. Vasc. Biol., 1995;15(9):1512-31) has developed a standardised classification of distinct plaques simplified by Fuster (Fuster *et al*, Circulation, 1994 Oct;90(4):2126-46) and reviewed by Corti *et al* (Ann N Y Acad Sci. 2001;947:181-95; and discussion on pages 195-8).

35 The process of atherosclerosis

The process of atherosclerosis can be viewed as a special type of chronic inflammation where monocytes adhere to the vessel wall and accumulate in the intima. In the presence of low-density lipoproteins (LDL) the monocytes are converted to activated macrophages, which take up lipoprotein particles and become

foam cells. This is followed by migration and proliferation of vSMCs **within** the arterial intima, leading to the great intimal expansion seen in atherosclerotic plaques.

The proliferation of the vSMCs is concomitant with a phenotypic modulation of the vSMCs from a contractile to a synthetic phenotype. In addition to proliferation, this phenotypic modulation leads to an increase in the production of extracellular matrix (ECM) molecules (Thyberg et al, Arteriosclerosis.

1990;10(6):966-90). The ECM is critical for the maintenance of vascular integrity and imparts tensile strength, viscoelasticity, elastic recoil and compressibility through the distinct properties of its constituents. Interactions between the ECM and vSMCs are mediated via cell surface receptors such as integrins. Integrins are heterodimeric molecules consisting of non-covalently bound alpha and beta subunits. These molecules mediate a broad distribution of interactions between cells and extracellular matrix components and affect a number of cellular responses including adhesion, migration, proliferation, differentiation, and apoptosis.

Alpha10

A newly discovered collagen-binding integrin, alpha10beta1, includes the integrin subunit alpha10 (Camper *et al.*, (1998) J. Biol. Chem. 273:20383-20389). The integrin is expressed on chondrocytes and shows a M_r of 160 kDa after reduction when isolated from bovine chondrocytes by collagen type II affinity purification.

Cloning and cDNA sequencing showed that it shares the general structure of other integrin alpha subunits. The predicted amino acid sequence consists of a 1167-amino acid mature protein, including a signal peptide (22 amino acids), a long extracellular domain (1098 amino acids) a transmembrane domain (22 amino acids), and a short cytoplasmic domain (22 amino acids). In contrast to most alpha-integrin subunits, the cytoplasmic domain of alpha10 does not contain the conserved sequence KXGFF(R/K)R. Instead, the predicted amino acid sequence in alpha10 is KLGFFAH. It is suggested that the GFFKR motif in alpha-chains are important for association of integrin subunits and for transport of the integrin to the plasma membrane (De Melker *et al.* (1997) Biochem. J. 328:529-537).

The extracellular part contains a 7-fold repeated sequence, an I-domain (199 amino acids) and three putative divalent cation-a binding site. The deduced amino acid sequence of alpha10 is 35% identical to the integrin subunit alpha2 and 37% identical to the integrin subunit alpha1. Sequence analysis has revealed that the alpha10 subunit is most closely related to the I domain-containing α subunits with the highest identity to alpha1 (37%), alpha2 (35%) and alpha11 (42%).

The role of integrins in atherosclerosis

Although several integrins are known to be present on normal vSMCs (Raines E.W., Int J Exp Pathol. 2000;81(3):173-82), little is known about the changes in integrin expression during atherosclerosis. It has been shown that injured (synthetic) vSMCs, in atherosclerosis, are able to modulate their integrin expression by switching from $\alpha 1 \beta 1$ to $\alpha 2 \beta 1$ and increase their production of fibrillar collagens, particularly collagen I (Skinner et al, Am J Pathol. 1994;145(5):1070-81.; Thyberg et al, Arteriosclerosis, 1990;10(6):966-90).

Cellular interactions are critical for the development of the atherosclerotic lesion at all stages of its evolution. The integrins, which are receptors for ECM-molecules such as collagens, have critical roles in the vSMCs communication with the extracellular matrix and modulating the phenotype of the vSMCs (Hynes R.O., Cell. 1992;69(1):11-25). Future therapy will be directed towards the modulation of these adhesive interactions mediated by the integrins, which in turn may arrest the development of the atherosclerotic plaque, limit plaque activation and attenuate the thrombotic response accompanying activation.

To-date, treatment of atherosclerosis is mainly performed by dietary adjustments and administration of cholesterol-lowering drugs to slow down or even halt the development of atherosclerosis. However, only limited success has been reported for regression of atherosclerosis. i.e. diminution of the atherosclerotic fatty plaque deposits.

It is thus highly desirable in the light of the aforementioned problems to develop means and methods for suitable therapeutic target molecules and potential diagnostic markers in atherosclerosis, and especially for the inhibition and regression of atherosclerotic plaques. In this respect, the present invention addresses this need and interest.

SUMMARY OF THE INVENTION

In view of the foregoing disadvantages known in the art when treating a mammal for atherosclerosis, or detecting atherosclerotic plaque, the present invention provides an antigen indicative of the presence of atherosclerotic plaque, namely the integrin $\alpha 10$ chain, suitable for preventing, treating or detecting atherosclerosis.

One object with the present invention is to provide methods for slowing or arresting progression and/or affecting regression of atherosclerotic plaque in a mammal.

Another object is to provide a method for detecting atherosclerotic plaque in a mammal.

Thus, the present invention provides a method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque in a mammal. The method comprises the steps of

- 5 a) administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal,
- b) scoring the progression and/or regression of atherosclerotic plaque, and
- c) correlating the scoring in b) above to slowing or arresting the progression and/or effecting the regression of atherosclerotic plaque.

10 Also, the present invention provides a method for treating atherosclerosis in mammals in the need thereof, the method comprising the step of administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal.

15 Further, the present invention provides a method for diagnosing a mammal who has or may be at risk of developing atherosclerosis, the method comprising the steps of

- a) determining the amount of integrin alpha10 chain in a biopsy obtained from the mammal.
- 20 b) scoring the amount of integrin alpha10 chain in said biopsy, relative to a control,
- c) correlating the amount obtained in step b) above with amounts obtained from the control to determine whether the mammal has or is at risk of developing atherosclerosis.

25 The method according to the invention may in further embodiments include wherein determining of the amount of integrin alpha10 chain further comprises contacting the sample with a binding agent having a binding site specific for said of integrin alpha10 chain.

Even further, the present invention include a method for detecting atherosclerotic plaque in a mammal, the method comprising the steps of

- 30 a) determining the amount of integrin alpha10 chain in a biopsy obtained from the mammal.
- b) scoring the amount of integrin alpha10 chain in said biopsy, relative to a control, and
- 35 c) correlating the amount obtained in step b) above with amounts obtained from the control to detect said atherosclerotic plaque in the mammal.

The methods according to the invention may in further embodiments include wherein the mammal is a human, or a rodent, such as a mouse or rat.

Uses of the integrin alpha10 chain for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque, for the preparation of a

composition for the treatment of atherosclerosis, for diagnosing atherosclerosis and for detecting atherosclerotic plaque are also provided.

SHORT DESCRIPTION OF DRAWINGS

Fig. 1 shows phases and lesion morphology in the progression of atherosclerosis (from: Corti et al 2001 Ann. N.Y Acad. Sci 947:181–198), and

Fig. 2 shows the detection of integrin alpha10 chain in an atherosclerotic plaque of a murine aorta. The top picture, A, shows the staining of the integrin alpha10 chain as a strong staining of the cell surface of a cell in the aortic plaque.

10 The middle photo, B, shows a specific blocking of the integrin $\alpha 10$ chain staining with a peptide of the integrin $\alpha 10$ chain, wherein almost all staining has been blocked by the peptide. The bottom photo, C, shows a control staining using secondary antibody only. No unspecific binding due to the secondary antibody, a donkey-anti-rabbit Cy3, is detected.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term "atherosclerotic plaque" refers to the build up of fatty plaque deposits within the wall of blood vessels.

20 The terms "rodent" and "rodents" refer to all members of the phylogenetic order *Rodentia*.

The term "murine" refers to any and all members of the family *Muridae*, including rats and mice.

25 The antigen

As revealed above, the present invention provides an antigen indicative of the presence of atherosclerotic plaque, namely the integrin alpha10 chain. The integrin alpha10 chain is known and publicly available at GenBank™/EBI Data Bank accession number AF074015. Thus, new uses and methods of said integrin alpha10 chain are disclosed in the present invention.

Accordingly, the present invention provides the use of the integrin $\alpha 10$ chain as a therapeutic target molecule and potential diagnostic marker in atherosclerosis to meet today's unmet need for methods that can be used in prevention, early detection and therapy of atherosclerosis.

A method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque

According to the invention, a method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque in a mammal is disclosed. Such

5 a method comprises the steps of

- a) administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal,
- 10 b) scoring the progression and/or regression of atherosclerotic plaque, and
- c) correlating the scoring in b) above to slowing or arresting the progression and/or effecting the regression of said atherosclerotic plaque.

The administration of an effective amount of a binding agent having a binding site specific for integrin alpha10 chain has to be evaluated in each case. The binding agent has to be administered over a range of doses to the mammal in the

15 need thereof, followed by assaying at various time points for (an) effect(s) of the agent on the atherosclerotic plaque. The effective dose of the binding agent can differ from mammal to mammal, and from patient to patient but in general includes amounts starting where clinical or pathological effects or other desired therapeutic effects on the atherosclerotic plaque occur, but below that amount where significant

20 undesirable side effects are observed. The dosage administered will depend on the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of the treatment, and the nature of the effect desired. The effective dosage can be modified by comparing the relative to in vivo potencies of the drugs and the bioavailability using no more than routine experimentation.

25 The binding agent is generally administered locally and/or systemically together with a pharmaceutically acceptable carrier, diluents and/or other additives. Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for

30 pharmaceutical active substances is well known in the art. Except insofar as any conventional medium or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The binding agent in an effective amount may be administered in any

35 convenient manner, either orally or parenterally such as by intravenous, intra-peritoneal, subcutaneous, rectal, implant, transdermal, slow release, intrabuccal, intracerebral or intranasal administration. For intracerebral administration, the binding agent needs to pass the blood brain barrier and may have to be chemically

For injectable use, sterile aqueous solutions (where water soluble) are generally used or alternatively sterile powders for the extemporaneous preparation of sterile injectable solutions may be used. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable binding agent can be brought about by the use in the binding agent of agents delaying absorption, for example, aluminum monostearate and gelatin.

20 Sterile injectable solutions are prepared by incorporating the binding agent in the required amount in the appropriate solvent with various amounts of the other ingredients enumerated above, as required, followed by sterilization by, for example, filtration or irradiation. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique, which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the binding agents are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The amount of active binding agent is such that a suitable effective dosage will be obtained.

Different forms of tablets, troches, pills, capsules and the like may also be used and contain other components as listed below.

35 The binding agent may in further embodiments be administered together with other agents, such as drugs, proteins, i.e. antibodies and enzymes, or peptides, i.e. polypeptides or oligopeptides, known to effect atherosclerosis, and particularly the atherosclerotic plaques. Such an additional other agent may be administered directly or indirectly linked to the binding agent or non-linked.

The binding agent according to the invention may be any protein, such as a

monoclonal or polyclonal antibody, or parts thereof; a polypeptide or an oligopeptide binding to the integrin alpha10 chain. The integrin alpha10 chain may also be in a heterodimer formed with the integrin beta1 chain. Thus, the binding agent may bind to both of the integrin chains, i.e. the alpha10beta1 heterodimer.

5 In further embodiments, the binding agent may bind any intracellular or extra cellular part of the integrin alpha10 chain.

The binding agent may in further embodiments bind to the I-domain and/or the splice domain of the integrin $\alpha 10$ chain.

The binding agent may also be any other molecule binding to integrin alpha10 chain, or said heterodimer, such as a molecule obtained from screening a chemical library.

The integrin alpha10 chain is expressed both intracellularly and extracellularly on cells in the atherosclerotic plaque, such as monocyte, macrophage, fibroblast or endothelial cell. Thus, further embodiments of the method according to the invention includes wherein the cell is a smooth muscle cell, and the binding site for the integrin alpha10 chain is intracellular and/or extracellular on the surface of such a cell.

In a specific embodiment, the binding site specific for the integrin alpha10 chain is on the cell surface of a smooth muscle cell.

20 The primary in vivo effect in a mammal is where the binding agent finds and localises the target, i.e. integrin alpha10 chain, on the cell surface of said cells in the atherosclerotic plaque. Still for detecting an atherosclerotic plaques, as described below, the intracellular expression of integrin alpha10 chain may be used as well.

25 *A method for treating atherosclerosis in a mammal in the need thereof*

According to the invention, a method for treating arteriosclerosis in a mammal in the need thereof is disclosed. The method comprises the step of

30 a) administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal.

Different embodiments including administration to the mammal, the binding agent as well as cells of interest are further described in the paragraph *A method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque.*

A method for diagnosing a mammal who has or may be at risk of developing atherosclerosis

According to the invention, a method for diagnosing a mammal who has or is at risk of developing atherosclerosis is included. The method comprises the steps of

- a) determining the amount of integrin alpha10 chain in a mammal,
- b) scoring the amount of integrin alpha10 chain in said mammal, relative to a control, and
- c) correlating the amount obtained in step b) above to determine whether the mammal has or is at risk of developing atherosclerosis.

5

In further embodiments, the method is a quantitative method, wherein the amount of integrin alpha10 chain is determined relative to the control in a quantitative manner.

In even further embodiments, the amount of the integrin alpha10 chain is determined in a biopsy from the mammal, such as a mouse, rat or human.

10

Further embodiments include wherein the biopsy is from any blood vessel, such as an artery.

Even further embodiments include wherein determining of the amount of integrin alpha10 chain further comprises contacting atherosclerotic plaque, or part of the mammal where the atherosclerotic plaque is suspected to be located, with a binding agent having a binding site specific for said integrin alpha10 chain. Said binding agent is further described in the paragraph *A method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque.*

15

Even further embodiments include wherein the scoring in b) above and correlating in c) above is a scoring and correlation to a particular disease stage of atherosclerosis.

20

A method for detecting atherosclerotic plaque in a mammal

According to the invention a method for detecting atherosclerotic plaque in a mammal is disclosed. Such a method comprises the steps of

25

- a) determining the amount of integrin alpha10 chain in a mammal,
- b) scoring the amount of integrin alpha10 chain in said mammal, relative to a control, and
- c) correlating the amount obtained in step b) above with amounts obtained from a control to detect the amount of atherosclerotic plaques.

30

In further embodiments, the method is a quantitative method, wherein the amount of integrin alpha10 chain in a mammal is determined relative to the control sample in a quantitative manner.

In even further embodiments, the amount of the integrin alpha10 chain is determined in a biopsy from the mammal, such as a mouse, rat or human.

35

Further embodiments include wherein the biopsy is from any blood vessel, such as an artery.

Further embodiments include wherein the amount of integrin alpha10 chain is determined in vivo.

Further embodiments include wherein the amount of integrin alpha10 chain is determined in vitro in a biopsy from said mammal, such as a mouse, rat or human.

Further embodiments include wherein the determining of the amount of integrin alpha10 chain is performed by immunohistochemical analysis in vitro. Such methods are known to the skilled man in the art, and further exemplified in the example below.

Further embodiments include wherein the determining of the amount of integrin alpha10 chain is done by any non-invasive method in vivo, such as any imaging method. Examples of such methods are Magnetic Resonance Imaging (MRI), Ultrasound, such as intravascular ultrasound (IVUS), Computed tomography, such as Electron Beam Computed Tomography (EBCT) and multislice tomographic scanning, as well as angiography. Any other suitable method known to the skilled man in the art may also be used.

Even further embodiments include wherein determining of the amount of integrin alpha10 chain further comprises contacting the sample with a binding agent having a binding site specific for said integrin alpha10 chain. Said binding agent is further described in the paragraph *A method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque.*

A method for modulating the expression of the integrin alpha10 chain.

The expression of the integrin alpha10 may also be modulated by gene targeting of the gene(s) encoding the alpha10 protein expression in a mammalian cell. The cell may be targeted in vivo or in vitro in a mammal, such as a human or a mouse. In vitro techniques for modulating expression of a specific gene, or group of genes are known to the skilled man in the art. In brief, the gene is normally targeted by a construct encoding a complementary nucleic acid sequence to the gene of interest to target. The whole gene may be targeted, as well as a part thereof. "A part thereof" here refers to a part of the gene that will regulate the expression, such as the promotor or other regulatory sequences of the gene, as well as the intron or exon sequences of the gene. Thus, the expression of the alpha10 protein may, due to the targeting at the gene level, be modulated to e.g. down regulate the expression of the protein.

Further embodiments include wherein the targeting of the gene is modulating the expression so as to upregulate the expression of the alpha10 chain protein. The up-regulation may be to a normal level of the gene. A normal level is herein intended to mean a level about what the cell in a specific mammal normally expresses in that particular cell type and activation stage of that cell type. This is sometimes referred to as the wild type expression as well.

Even further embodiments include in vivo modulation of the integrin alpha10 chain protein expression. Such in vivo modulation may as well be due to a

regulation at the gene level due to targeting of the gene in vivo. Thus, the expression of the alpha10 protein may, due to the targeting at the gene level, be modulated to e.g. down regulate the expression of the protein in vivo.

Further embodiments include wherein the targeting of the gene is modulating the expression so as to up-regulate the expression of the alpha10 chain protein in vivo.

According to the invention, a method for modulating the expression of the integrin alpha10 chain in a cell in an atherosclerotic plaque is disclosed. Such a method comprises the steps of

- 10 a) providing a construct targeting the integrin alpha10 chain gene, parts thereof or regulatory sequences to the gene,
- b) contacting the cell in an atherosclerotic plaque in vivo or in vitro with said construct,
- c) transforming the cell in vivo or in vitro,
- 15 d) optionally selecting the transformed cell in vivo or in vitro,
- e) detecting the protein expression of the alpha10 chain protein,
- f) correlating the protein expression of the alpha10 chain protein to the expression before providing the construct in a) above, thereby evaluating the modulation of the expression of the integrin alpha10 chain protein.

20 The method above may be used as a gene therapeutic method for modulating the expression of the integrin alpha10 chain protein. Such a use may be to suppress, prevent, increase or decrease the in vivo expression in a mammal, such as a human, mouse or rat, in the need thereof. By re-introduction of nucleic acid, such as deoxynucleic acid (DNA), ribonucleic acid (RNA), peptidenucleic acid (PNA) or
25 mixtures thereof, the activity may be modulated and e.g. an absence or over-expression of the integrin alpha10 chain protein partially or fully compensated.

Still another use of the method according to the invention is to prevent, inhibit, alleviate or reverse activity in atherosclerosis.

30 *The mammal*

For the methods and uses disclosed, the mammal may in further embodiments be a human.

Still further embodiments include wherein the mammal is a rodent, such as a rat or mouse or any other member of the family Muridae.

35 In some of the methods and uses, a mammal in the need thereof is disclosed. Such a mammal, e.g. a human or mouse, may be any mammal showing early signs of atherosclerotic plaque, fully developed atherosclerotic plaque, or being predisposed to or in the risk of forming an atherosclerotic plaque.

Different conditions may lead to atherosclerosis in a mammal. Thus, further

embodiment include wherein the atherosclerosis in a mammal is caused by conditions such as type 1 diabetes, ApoA1 deficiency, a cardiovascular disorder, e.g. coronary artery disease (CAD), systemic lupus erythematosus (SLE), acute ischemia, chronic ischemia or Sjögren's syndrome.

- 5 Other risk factors for atherosclerosis include hypertension, elevated levels of low density lipoprotein, reduced levels of high density lipoprotein, cigarette smoking, diabetes mellitus, obesity, male sex, elevated homocysteine levels, chlamydia pneumoniae infection, and family history of premature atherosclerosis. Thus, even further embodiments include wherein the mammal in the need thereof is
- 10 a mammal being exposed to different risk factors involved in atherosclerosis, such as the risk factors mentioned above.

Uses according to the invention

- According to the invention, several uses of the integrin alpha10 chain are
- 15 disclosed.

A use of the integrin alpha10 chain for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque is included.

- Further, a use of the integrin alpha10 chain for the preparation of a composition for the treatment of atherosclerosis is included. Such a treatment of
- 20 atherosclerosis is for a mammal in the need thereof. The mammal, e.g. a human or mouse, may be any mammal showing early signs of atherosclerotic plaque, fully developed atherosclerotic plaque, or being predisposed to or in the risk of forming an atherosclerotic plaque.

- Further, a use of integrin alpha10 chain for diagnosing atherosclerosis is
- 25 disclosed.

Even further, a use of integrin alpha10 chain for detecting atherosclerotic plaque is included.

EXAMPLES

30

Example 1 Detection of the integrin alpha10 chain in aortic atherosclerotic plaque

Objective

- The objective of this example is to describe detection of the integrin alpha10
- 35 chain in aortic atherosclerotic plaque.

Material

Murine aortic plaque sections were used. The sections were cut on a cryostat at a thickness of 5µm onto Superfrost Plus slides (Menzel-Gläser, Histolab). The cut sections were then stored at -20°C.

5 *Detection of the aortic plaques*

Before staining, the frozen sections were allowed to thaw to room temperature for about 30 minutes.

The sections were fixed in acetone for 10 minutes at -20°C, and then treated with hyaluronidase (2mg/ml, Sigma) for 30 minutes at 37°C.

- 10 The sections were blocked with 4% donkey serum (Harlan) diluted in PBS for 30 minutes at room temperature.

During the blocking step, an anti-alpha10 antibody was incubated with an alpha10 peptide, i.e. the blocking peptide, at different concentrations, e.g. 0.01, 0.1, and 0.5 mg/ml, for 30 minutes at 4°C.

- 15 The sections were then incubated with rabbit anti-mouse alpha10, in 1:100 dilution, and a fraction of the preincubated rabbit anti-mouse alpha10 and the blocking peptide in a 1:100 dilution. The sections were incubated for 75 minutes at room temperature.

- 20 After a 15-minute wash in PBS, the sections were incubated with donkey anti-rabbit Cy3 (IgG, 1:100, from Jackson) for 60 minutes in room temperature.

After a final 15-minute wash in PBS, the sections were mounted with Vectashield (Vector Labs) and photographed under a fluorescent microscope.

25 *Results and discussion*

In figure 2, the result of the staining to detect the integrin alpha10 expression in the aortic plaques is shown. The top picture, A, shows the staining of the integrin alpha10 chain as a strong staining around the outside of the cells in the aortic plaque. The middle photo, B, shows a specific blocking of the integrin alpha10 chain staining with a peptide of the integrin alpha10 chain, wherein almost all staining has been blocked by the peptide. The bottom photo, C, shows a control staining with the secondary antibody used. No unspecific binding due to the secondary antibody, a donkey anti-rabbit Cy3, is detected.

CLAIMS

1. A method for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque in a mammal, the method comprising the steps of
 - 5 a) administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal,
 - b) scoring the progression and/or regression of atherosclerotic plaque, and
 - 10 c) correlating the scoring in b) above to slowing or arresting the progression and/or effecting the regression of said atherosclerotic plaque.
2. The method according to claim 1, wherein the cell surface is the cell surface of a smooth muscle cells.
- 15 3. A method for treating atherosclerosis in mammals in the need thereof, the method comprising the step of administering to the mammal an effective amount of a binding agent having a binding site specific for integrin alpha10 chain on the cell surface of said mammal.
- 20 4. A method for diagnosing a mammal who has or may be at risk of developing atherosclerosis, the method comprising the steps of
 - a) determining the amount of integrin alpha10 chain in a mammal.
 - b) scoring the amount of integrin alpha10 chain in said mammal, relative to a control,
 - 25 c) correlating the amount obtained in step b) above with amounts obtained from the control to determine whether the mammal has or is at risk of developing atherosclerosis.
5. The method according to claim 3, wherein the determining is performed in vivo.
- 30 6. The method according to claim 3, wherein the determining is performed in vitro.
7. The method according to any of claims 3-6, wherein determining of the amount of integrin alpha10 chain further comprises contacting the integrin alpha10 chain with a binding agent having a binding site specific for said of integrin alpha10 chain.
- 35 8. A method for detecting atherosclerotic plaque in a mammal, the method comprising the steps of

- a) determining the amount of integrin alpha10 chain in a mammal,
 - b) scoring the amount of integrin alpha10 chain in said mammal, relative to to a control, and
 - c) correlating the amount obtained in step b) above with amounts obtained from the control to detect said atherosclerotic plaque in the mammal.
- 5
9. The method according to claim 8, wherein the determining is performed in vivo.
 10. The method according to claim 8, wherein the determining is performed in vitro.
 - 10 11. The method according to any of claims 8-10, wherein determining of the amount of integrin alpha10 chain further comprises contacting integrin alpha10 chain with a binding agent having a binding site specific for said of integrin alpha10 chain.
 - 15 12. The methods according to any of claims 1-2, 3, 4-7, 8-11, wherein the mammal is a human.
 13. The methods according to any of claims 1-2, 3, 4-7, 8-11, wherein the mammal is a mouse or rat.
 - 20 14. Use of integrin alpha10 chain for slowing or arresting the progression and/or effecting regression of atherosclerotic plaque.
 - 25 15. Use of integrin alpha10 chain for the preparation of a composition for the treatment of atherosclerosis.
 16. Use of integrin alpha10 chain for diagnosing atherosclerosis.
 - 30 17. Use of integrin alpha10 chain for detecting atherosclerotic plaque.

An antigen indicative of the presence of atherosclerotic plaque is provided. The antigen is used in a method for slowing or arresting progression and/or effecting regression of atherosclerotic plaque in a mammal, comprising administering to the mammal a binding agent having binding sites specific for said antigen of said mammal. Also included are methods for diagnosing a mammal who has a risk of developing atherosclerosis, comprising determining the amount of said antigen. Moreover, uses of said antigen for detection and diagnosing atherosclerosis is provided.

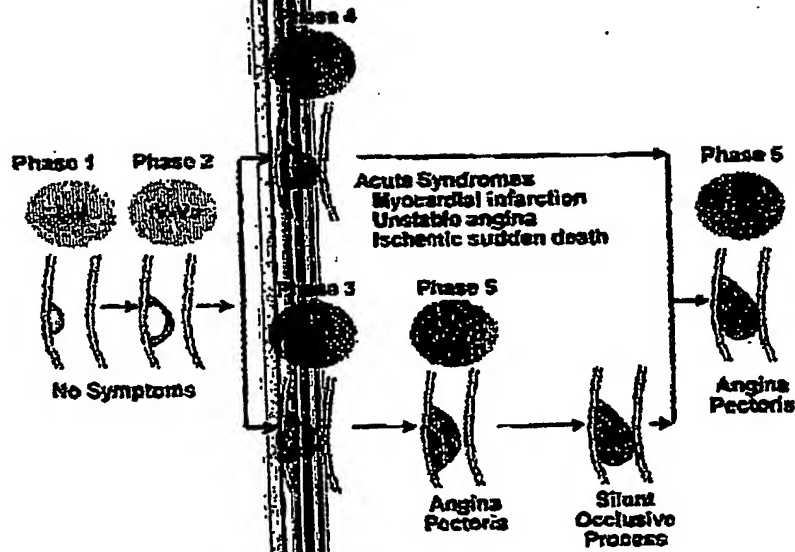


Figure 1

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Patentbeskrivning

Fig. 17

Fig. 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100

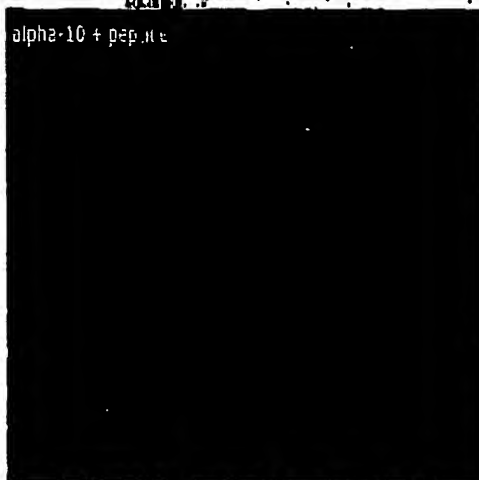
A.

alpha-10



B.

alpha-10 + peptide



C.

control

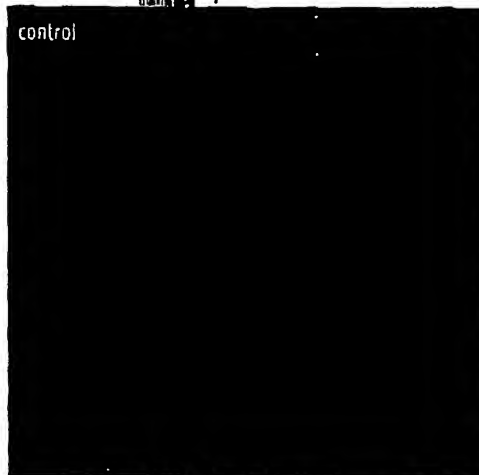


Figure 2

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